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Block copolymer micelles as vehicles for drug delivery

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There is a wide-spread consensus that characteristics of drug vehicles determine the applicability of the site-specific delivery of drugs. This article focused on the promising features of block copolymer micelles as drug vehicles mimicking the natural carrier-systems with supramolecular structures (i.e. viruses and lipoproteins). Considerable discussions are made on the physicochemical characteristics of polymeric micelles in aqueous milieu with shedding light on earlier works done in the field. Advantageous features of polymeric micelles as drug vehicles are summarized as: (1) formation of environmentally-separated microcontainer of drugs through supramolecular assemblage, (2) installation of anchoring moiety on the surface, (3) duration in the biological compartment and (4) programmable chronological stability. Then, our recent work concerning polymeric micelles with anti-tumor activity is presented to demonstrate these advantageous features of polymeric micelles. Worth noticing is that higher anti-tumor activity was achieved by adriamycin-conjugated micelles compared with parental adriamycin, indicating that a considerable improvement in cancer chemotherapy is feasible by the use of appropriate vehicle systems.

Keywords: Polymer micelle; Block copolymer; Poly(ethylene oxide); Site-specific delivery; Anti-cancer drug

Introduction

There has been a strong impetus for developing efficient systems for site-specific delivery of drugs by the use of appropriate vehicle systems [1–3]. Especially, tremendous efforts have been devoted to searching for and designing the so-called pilot molecules, including antibodies and ligand molecules to specific receptors. However, it becomes clear that, even though the smart pilot molecules are equipped on vehicles, efficient accumulation of drug to the target-site is not always found to be possible. This is due to the non-specific elimination, and enzymatic or non-enzymatic degradation of vehicle systems. In vivo fate of vehicle systems after the intravenous injection is schematically shown in Fig. 1. Vehicles have a high-incidence of excretion through kidneys as well as of hepatic elimination. Recognition by reticuloendothelial systems (RES) is known to be a main reason for the removal of vehicles from the blood compartment. And, if the target sites are located outside of the blood com-

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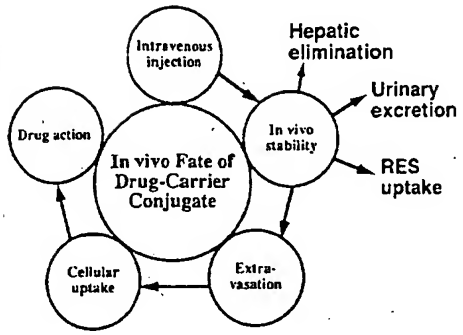

Fig. 1. Schematic itinerary of drug-carrier conjugate (vehicle systems) dosed intravenously.

partment, as in the case with solid tumors, the vehicle is required to have a capability of penetrating through the vessel wall, unless a sustained-release mechanism is adopted. It is after the extravasation that pilot molecules on the vehicle become active through the binding with cells expressing marker molecules. This intricate itinerary of vehicle systems shown in Fig. 1 strongly indicates the primary importance of the development of an ideal vehicle system with 'stealth' property. By using this type of stealth vehicle, we may change the pharmacokinetics and biodistribution of drugs, and even accumulate them, on some occasions as will be described later, to the vicinity of target site with a simple diffusion process. Pilot molecules, if any, might further facilitate the retention and the cellular uptake of the vehicle at the target site.

It is to be noted that there is a sophisticated example of a stealth vehicle existing as biological entities; i.e., viruses. As well known, the virus is a natural vehicle for nucleic acids, which in naked form might promptly be degraded in the body compartments. The reason for the stability of viral gene is that it is protected from te outer environment through the formation of supramolecular structures [4,5]. The architecture of the spherical virus is typified by the core-shell structures composed of the macromolecular sub-units. The inner core is DNA or RNA, which is protected by an outer shell called 'capsid' constructed of sub-units of globular proteins. The adenovirus is a typical example of this type of spherical viruses. Many of the eukaryotic viruses further coat their capsids with an outer-layer composed of lipid-bilayer membranes (enveloped capsids). These viral architectures are formed through self-assembly of biopolymers with a requirement of minimizing free energy. Worth noticing is that many animal viruses range in size from 20 to 100 nm [6]. This may contribute to the avoidance of RES uptake, since RES recognition is believed to be considerably lowered for particles less than ca. 100 nm. Further, a virus is hardly excluded from kidney, because its size is large enough to avoid renal excretion. Consequently, viruses are able to stably circulate through the blood compartment, and some viruses successfully extravasate to reach target sites, where they attach and invade into host cells.

Viral architectural features are as follows: (1) formation of microcontainer to protect transporting substance (in this case viral gene) from the outer environment (core-shell structure); (2) stealth property in a host entity (biocompatibility); (3) installing pilot molecules on the surface (spatial recognition); (4) chronological action through structuring and destruction of macromolecular assemblage (temporal structure).

Particularly important is the dynamic or temporal feature of viral structure. It promptly collapses during the invasion process into a host cell to transport the viral gene inside the host cell. In a replication process, viral structure is formed in the host cell through a self-assembly manner.

There are other examples of natural vehicles typified by their core-shell structures: i.e., lipoproteins. The lipoproteins are lipid-protein complexes with hydrophobic core, and work as the transporters of cholesterol and lipids in the blood. There are four main classes in lipoproteins with varying size, i.e., high density lipoprotein (HDL, size range: ~10 nm), low density lipoprotein (LDL, size range: ~20 nm), very low density lipoprotein (VLDL, size range: 30~90 nm) and chylomicrons (size range: 10~100 nm) [7]. Worth noticing is that size range of lipoproteins is about the same range as viruses. Further, in principle, lipoproteins have the aforementioned features listed for viral architecture. Both virus

and lipoproteins are regarded as sophisticated nanoscopic vehicles in biological systems.

Self assemblage of amphiphilic block copolymers

Nanoscopic vehicles having a microcontainer separate from the outer environment are promising for site-specific delivery of drugs as well as of external genes. Indeed, promising results are reported for the use of natural vehicles, i.e., viruses and lipoproteins for this purpose [7–9], yet the tailoring process is rather complex and choice of incorporated substances is considerably restricted. Conceptual features of these natural vehicles is the self-assemblage of macromolecules to form supramolecular structure (core-shell structure) in nanoscopic size. This feature might be modeled in a much simpler manner through the tailoring of synthetic macromolecules with amphiphilic properties.

It is well-known that block copolymers in a selective solvent (a good solvent for one block but a nonsolvent for the other) form a micellar structure through the association of the insoluble segments as schematically shown in Fig. 2 [10,11]. This type of multimolecular micell with

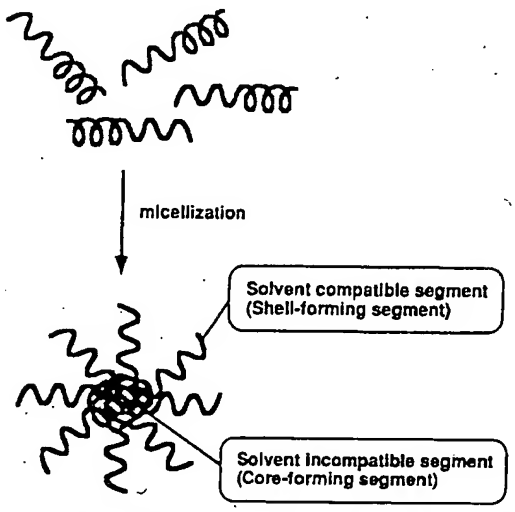

Fig. 2. Multimolecular micellization of block copolymers in a selective solvent.

core-shell architecture was first verified by Plestil and Baldrian through small-angle X-ray scattering (SAXS) measurement of polystyrene/polybutadiene/polystyrene ABA block copolymer in ethyl methyl ketone, which is a selective solvent for polystyrene [12]. Formation of polymeric micelles in organic solvent systems has so far been studied intensively to characterize the properties of these micelles in terms of critical micelle concentration (cmc), micelle shape, size and size distribution by using a variety of techniques, including light scattering, sedimentation, SAXS, neutron scattering, gel permeation chromatography and electron microscopy [10,11]. However, there are only a few known examples of block copolymers forming micellar structure in aqueous milieu. A series of poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) block copolymers, known as Pluronics or poloxamer in commercial name, is the one for which considerable efforts have been devoted to clarify its association behavior in aqueous systems [13–16].

It is to be noted that poloxamers show a considerably low toxicity [17], allowing them to be utilized in the pharmaceutical field. Schmolka suggested, in his review, the feasibility of poloxamer as a solubilizer of biologically-active substances, including cholesterol and fluorocarbon [18]. In this sense, the amphiphilic nature of poloxamer may mimick the function of lipoproteins, natural vehicles for lipophilic substances, although the association of poloxamer molecules to multimolecular micelle is still doubtful in physiological conditions as will be described later. Recently, Kabanov et al. reported a considerable improvement in in vivo activity of a drug (haloperidol) associated with poloxamer [19].

Although the cmc is the most fundamental parameter to characterize thermodynamical stability, at least static, of the mielle system, doubts have been raised as to the value for the cmc of poloxamers measured by different techniques [13–16,18]. This might be attributed to the change in micelle structure from monomolecular (intramolecular) association to multimolecular

association with an increase in the concentration. Prasad et al., who investigated the problem by surface activity measurement as well as by dye solubilization, reported that the cmc for multimolecular micelle formation was in the range between 0.1 to 2.4 wt% depending on the ratio of hydrophilic poly(ethylene oxide) segment to hydrophobic poly(propylene oxide) segment in the chain [13]. Turro et al. reported an even higher cmc, 10 wt%, measured by fluorescence probe technique [15]. These results arouse the question of multimolecular micelle formation by these poloxamers in an in vivo situation where the dosed micelles may be infinitely diluted. Reasons for this relatively high cmc of multimolecular micelle formation by poloxamers are considered to be the high mobility of the poly(propylene oxide) segment at physiological temperature (i.e., low glass transition temperature) as well as the lower level of the interfacial free energy between water and poly(propylene oxide) chain. However, it is to be noted that the polydispersed feature (or open association) of poloxamer association changes to essentially monodisperse (or closed association) by increasing the temperature toward the cloud point or lower critical solution temperature (LCST) of the poloxamer [16], indicating the stabilization of the micelle state with minimum free energy by an increase in the interfacial free energy between water and poly(propylene oxide) chain. In summary, the poloxamer system surely has a solubilizing effect typical of biologically active substances with a hydrophobic nature and, with an advantage of less toxicity, it might be useful as a pharmaceutical adjuvant; although multimolecular micelle formation with core-shell structure is uncertain especially under the physiological conditions.

The approach of tailoring block copolymer micelles as drug vehicles was initiated by Ringsdorf et al. along with the concept of mimicking lipoprotein structure [20]. They prepared an AB block copolymer in which the A-block is hydrophilic poly(ethylene oxide), and the B-block consists of biodegradable poly(L-lysine) with a considerable substitution of the ϵ-amino groups with hydrophobic palmitoyl groups as well as with sulfidoderivatives of cyclophosphamide(CP) via hydrophobic alkyl spacer. The relatively labile CP-sulfidoderivatives can be stabilized due to their insertion into the hydrophobic environment of the block copolymer, which was suggested through the retardation in the liberation rate of the active metabolite 4-hydroxy-cyclophosphamide [20]. Micelle formation of this block copolymer, with both hydrophilic and hydrophobic regions, was indicated through the solubilization study of a dye, Sudan Red 7B [21]; the effect observed was similar to low molecular weight micellar analogues. Judging from the inflection point of solubilization, the cmc for micelle formation might be in the range of 0.1 ~ 0.5 wt.%, which is about the same as with poloxamer system. A preliminary in vivo studies for this cyclophosphamide-block copolymer conjugate showed that it had a prolonging effect on the life span of L1210 tumor-bearing mice [22].

Aforementioned examples of amphiphilic block copolymers have a hydrophobic moiety with a considerably flexible or mobile nature, which may form a core in a liquid state. However, it has become clear that amphiphilic block copolymers with glassy hydrophobic segments form a considerably stable structure of multimolecular micelles. Polystyrene (PSt) is a well-known hydrophobic polymer with glassy properties at physiological temperature (T_g=100°C). Pioneering work for the poly(ethylene oxide) (PEO)-PSt block copolymer was done by Nakamura et al. in the middle of 70s to estimate the surface activity and dye solubilizing properties of an aqueous solution of PEO-PSt block copolymers [23]. Multimolecular micelle formation of PEO-PSt block copolymer in aqueous solution was verified by Riess et al. by the use of static light scattering, from which the apparent molecular weight and the association degree of the micelles were estimated [24].

Further progress in photon correlation spectroscopy (dynamic light scattering) has enabled determination of the precise diameter of PEO-PSt block copolymer micelles in aqueous solu-

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tion [24,25]: PEO-PSt diblock and triblock copolymers, with M_n values ranging from 8500 to 29 000, were reported to form multimolecular micelles in water with hydrodynamic radii ranging from 9 to 22 nm [26]. Further, extremely low cmc (ca. 1-5 mg/l) were calculated for these micelles through a fluorescence probe study using pyrene [27,28]. This result indicates that the system can retain a micellar structure even in a very diluted condition, featuring polymeric micelles which may be useful as drug vehicles.

From the standpoint of using polymeric micelles as drug vehicles, the dynamic stability of the micelle is a more important property than the static stability indicated by the cmc. Once drug vehicles are introduced in the blood compartment, they are virtually infinitely diluted to conditions below cmc. Then, micelles become thermodynamically unstable, and they have to relax or, in the open system, to decay into constituent free chains (unimers). Worth noticing is that release rate of unimers from the micelle is expected to be quite slow for micelles with cores associating through strong cohesive forces or having a glassy structure. This feature allows polymeric micelles to circulate through the blood compartment and to reach the target site before decaying into unimers. In turn, one may construct a polymeric micelle system with a programmed decaying property by tailoring the chemical structure of the micelle-core. As will be described in the next section, we have recently succeeded in developing a stable multimolecular micelle system with a drug-binding inner core [29,30] and have verified its excellent utility as the vehicle for targeting therapy [31,32]. There is no doubt that the stable core-shell architecture is a great enabling the polymeric micelle to install hydrophobic drugs into the inner core by physical entrapment or covalent binding, resulting in an increased solubilization of the hydrophobic drug under physiological conditions. Further, it was clarified through our study that polymeric micelle can achieve a prolonged half life in the blood compartment, suggesting its promising feature as a stealth vehicle like viruses and lipoproteins.

Structural design of micelle-forming polymeric drugs

In this section, our design concept for micelle-forming polymeric drug, adriamycin-conjugated poly(ethylene oxide)-poly(aspartic acid) block copolymer (PEO/PASP(ADR)), is described. PEO/PASP(ADR) (Fig. 3) was prepared by conjugating adriamycin (ADR), a hydrophobic anti-cancer drug, to pendant carboxyl groups of poly(ethylene oxide)-poly(aspartic acid) block copolymers (PEO/PASP) [29,30,33]. We focused on the system by which micelle formation is mainly driven through hydrophobicity and the cohesive force of the conjugated drug itself, because both high loading capacity and high stability can simultaneously be achieved in this system. Further, another advantage concerning carrier excretion might be expected in this system (this subject will be elaborated in the next Section). Although ADR has been shown to be very effective in cancer chemotherapy [34], the drug exerts severe toxicity on heart and bone marrow. A promising strategy to prevent toxic side-effects of ADR is conjugation to the polymeric carrier systems [35]. Based on this strategy, conjugation of ADR to polymeric carriers with pendant carboxyl groups has been done by several research groups [36,37]. Limited conjugation was possible (up to ca. 10 mol% of carboxyl residues) before precipitate formation due to the strong hydrophobicity and cohesive force (mainly, aromatic π-stacking) of ADR mole-

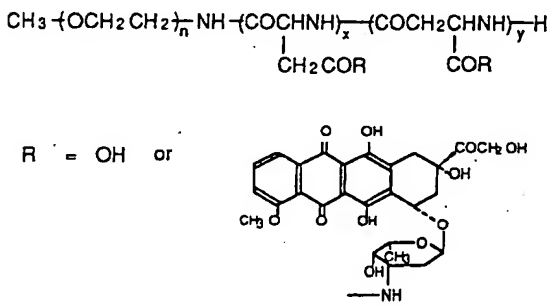

Fig. 3. Structural formula of adriamycin-conjugated poly(ethylene oxide)-poly(aspartic acid) block copolymer (PEO/PASP(ADR)).

cules. In turn, this segregative property of ADR from water favors the micelle formation of the PEO/PASP(ADR) system. Conjugation via pendant carboxyl residues of PASP segment and the amino group in the daunosamine moiety of ADR was successfully achieved, using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as a coupling agent, without precipitation for PEO/PASP with the length of the both segments ranging from 1000 to 12 000 in M_n values for PEO and from 10 to 80 repeat units for PASP segments [30,38]. The substitution degree of the carboxyl residues with ADR can be controlled in a wide range, and almost quantitative substitution was achieved for PEO/PASP (12-20), a block copolymer with PEO of $M_n = 12\,000$ and PASP with 20 repeat units of Asp. Wt% of conjugated ADR per polymer chain, a practical parameter of drug installing capacity, as high as 77% was achieved. ADR molecules were directly coupled onto PASP segments via amide linkage, so that they might not be released from the conjugate through bond cleavage at least in the extracellular condition with neutral pH. We designed the system to be in an active form only after the interaction with tumor cells at the target site. Several mechanisms have been proposed to explain the antitumor activity of ADR [35,39] which include: (1) intercalation into DNA, (2) interaction with plasma membranes and (3) the formation of free radicals through bioreductive activation. Although release of free ADR from the conjugate is essential for the first mechanism, it is not necessarily the case if second or third mechanisms play a crucial role in the cytotoxicity.

PEO/PASP has many advantageous features as the parent block copolymer in polymeric micelle drugs. Firstly, the synthetic route of various block copolymers of PEO and poly(amino acids), including PEO/PASP, has been well established through our earlier studies [29,33,40,41], and high reproducibility is guaranteed for their synthesis. Primary amino groups situated at the terminal of α-methyl-ω-amino-poly(ethylene oxide) was utilized to initiate the ring-opening polymerization of β-benzyl L-aspartate *N*-carboxyanhydride to obtain the block copolymer of PEO and poly(β-benzyl L-aspartate) (PBLA), followed by the debenzylation of this block copolymer under alkaline condition to prepare PEO/PASP. Through this alkaline hydrolysis, 75 mol% of α-amide linkages in poly(amino acid) segment were isomerized to β-amide bonds via intramolecular imide formation [30], although no main chain scission took place. As aforementioned, the PASP segment in the block copolymer serves as the drug-conjugating moiety to form the inner-core of the micelle. Further, it has an advantage of degrading into aspartic acid which can enter the natural metabolic cycle. The PEO segment serves as the outer shell of the micelle to protect the drug reservoir (inner core) from the outer environment. It is known to be non-toxic, and has been utilized in protein modification to decrease immunogenicity of intact proteins and to prolong the half life of the proteins in the blood compartment [42–44]. High flexibility as well as high degree of hydration are unique physical features of the PEO chain in the aqueous entity, and it is recognized that these features contribute PEO-attached surface to become protein-resistant (the surface showing minimizing protein adsorption) probably through steric exclusion mechanism [45]. This minimized interaction with protein is favorable to utilize PEO as outer-shell of the micelle. However, one should keep in mind the strong hydrogen-bonding character of PEO chains [46]. It may provoke considerable interaction with biological components under certain circumstances such that hydrated water molecules are replaced with components showing a strong hydrogen-bonding property. This mode of interaction might become important at the interface of PEO micelles with target cells as will be described later.

Micelle formation of PEO/PASP(ADR) was directly verified through the measurement of the hydrodynamic radius by dynamic light scattering [30,31,38]. Although the average diameter of the micelle varied somewhat with length and weight fraction of both segments, particles with an average diameter in the range of 15-60 nm were observed [38]. Further, the distribution is

essentially unimodal in terms of number-averaged scale. A small fraction of larger-size particles (ca. 100 nm) in weight-averaged scale was observed, which might be attributed to a micellar association similar to that reported for the PEO/PSt system in water by Winnik et al. [26,28]. They attributed this secondary association of the micelle to the attractive interactions between interpenetrated PEO chains of the associated micelles. This attraction of PEO chains at their high local concentration is supported by the increased values of Flory-Huggins χ-parameter (parameter for excess chemical potential) between PEO and water, exceeding 0.5 with increased volume fraction of PEO [47]. Worthy of mention is that the size range of the micelles composed of PEO/PASP (ADR) approximately corresponds to that of viruses and lipoproteins; suggesting the feasibility that these micelles can escape RES recognition. Core formation of ADR-conjugated segments was demonstrated through fluorescence measurement where conjugated-ADR showed strong quenching of its fluorescence due to the dense packing into the collapsed core of the micelle [31], as well as through lack of a ^{1}H-NMR signal from the ADR-conjugating segment. Because of this micelle formation, PEO/PASP (ADR) exhibited excellent water solubility, irrespective of the large quantity of incorporated ADR, and can be concentrated without precipitation to 20 mg ADR equivalents/ml in saline, the concentration where even the solution of free ADR turns to a gel state [31]. Further, it was possible to be stored in lyophilized form without losing its water solubility in the redissolving process [30,31]. Table 1 summarizes advantageous pharmaceutical properties of polymeric micelles, indicating their promising feature as vehicles for drugs, including ADR. It is to be noted that polymeric micelles can be sterilized by simple filtration, affording a great merit in practical handling. Further, as expected, drug molecules in the core are stabilized even in the aqueous solution form, due to the segregation from water molecules. This was confirmed through the prolonged stability of the ADR moiety in the micellar solution, as judged by the decrease in the characteristic band intensity (485 nm) of the anthracycline ring in ADR [31].

TABLE 1

Advantageous pharmaceutical properties

1. High water solubility core (hydrophobic) – shell (hydrophilic) structure
2. High carrying capacity of hydrophobic drug inner core as drug micro-reservoir
3. Simple sterilization by microfiltration micelle size: ca. 50 nm
4. Prolonged storage in freeze-dried state
5. Low viscosity

Pharmacokinetics and biodistribution of micelle-forming polymeric drugs

Biodistribution of ADR-conjugated micelle (PM-ADR) was determined by the intravenous injection of the micelle labelled with radio-isotope [32]. Aglycone (anthracycline ring) of ADR molecule in the micelle was successfully labelled with ^{125}I using the chloramine-T method. Then, the labelled micelles were intravenously injected into mice to estimate the stability and biodistribution. ADR and its conjugate with conventional polymeric carrier, poly(aspartic acid), were reported to undergo quick removal from the blood compartment [48–51]. Contrary to these conventional data, a dramatic improvement in pharmacokinetics was obtained for ADR conjugating into PEO/PASP. In terms of the amount of ADR equivalent in the blood at 1 h after injection, PEO/PASP (ADR) achieved values of more than 70-times higher than free ADR. This result strongly suggests that PEO/PASP (ADR) retains its micellar structure even in the blood compartment. This was further supported by the fact that ADR binding to the homopolymer of PASP gave no improvement in the

pharmacokinetic behavior [48]. Also, micellar structure was observed for plasma samples taken 1 h after injection of PEO/PASP (ADR) by gel permeation chromatography [32]. ADR equivalent in the micellar form was determined from the area of the chromatogram, and was found to be approximately equal to the value in the whole blood measured from radioactivity.

Further worthy to mention is the considerably high initial concentrations in blood for PEO/PASP (ADR) [32]. Namely, at least 60% of total dose of ADR equivalent was estimated to be appeared in the blood for PEO/PASP (ADR). This is quite a large value considering the very large distribution volume of parental ADR which is known to be due to non-specific adsorption to tissues [50]. This drawback is obviously improved in the PEO/PASP (ADR) system due to the increased solubility and the decreased tissue-adsorptivity of the micelle with a PEO outer-shell. The steric exclusion property together with the highly hydrated structure of PEO chains might play a role in the minimization of interactions with biological components. However, it is to be noted that the micelle stability is crucially affected by several factors, including chain lengths and weight ratio of both segments in the block copolymer as well as the content of ADR moieties along the polymer chain [38]. Programmed decay of the micelle through the release of the unimer is feasible by regulating these factors. Our recent study on the long-term pharmacokinetic behavior of micelles with varying composition demonstrated that the fate of the micelle in the blood compartment was indeed regulated through micelle stability which is mainly determined by wt-% and the chain length of PEO segments [52].

Non-specific accumulation to major organs, including heart, lung and liver, was considerably lowered by conjugating ADR to PEO/PASP [32], suggesting a promising feature of PEO/PASP (ADR) to be lowered toxic side effects compared with free ADR. In summary, these drastic improvements in pharmacokinetic behavior and biodistribution of ADR conjugated to PEO/PASP strongly suggest that the polymeric micelles with appropriate structures can surely behave as stealth vehicles in the living body. Further, the prolonged circulation in the blood compartment strongly suggests that the polymeric micelles have the capability of avoiding RES uptake. The size range of the polymeric micelles (ca. 20-50 nm) is considered to contribute to the avoidance of uptake by cells of the RES systems. Also, the highly swollen and flexible shell of the PEO may play a crucial role in diminishing the recognition of RES cells toward the polymeric micelles. It is a unique feature of the polymeric micelle to form a soft shell/hard core structure in which the collapsed core of less than 10 nm is surrounded by a hydrated soft shell with an elastic property.

Anti-cancer activity of polymeric micelles with an adriamycin-conjugated inner core

In vivo anti-cancer activity of PEO/PASP (ADR) was first determined for P388 mouse leukemia [31]. The mice, i.p. inoculated with P388 mouse leukemia cells, were given single i.p. injection of free ADR or PEO/PASP (ADR). Although the optimum dose given the maximum median life-span over controls (T/C %) was increased ca. 10 times, PEO/PASP (ADR) gave even better survival (T/C: 490% at 200 mg/kg) compared to free ADR (T/C: 305% at 15 mg/kg). PEO/PASP (ADR) lowered toxicity judging from the body weight change of the mice inoculated with free ADR or PEO/PASP (ADR) at the doses giving the maximum T/C values. In sharp contrast with the continuous loss of weight for the mice dosed with free ADR, a gain in the weight for the mice treated with PEO/PASP (ADR) was observed. Recently, the low toxicity of PEO/PASP (ADR) was further confirmed by the measurement of kidney and liver function of the mice given i.v. injection of drugs as well as from the pathological condition of the major organs [32]. Judging from these assays, the toxic score of ADR equivalent in PEO/PASP (ADR) is estimated to be as low as 1/20 that of free ADR. This drastic decrease in systematic toxicity for PEO/PASP (ADR) is consistent with the

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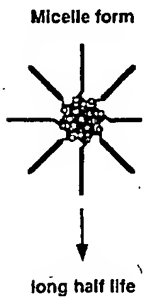

Fig. 4. Feasible
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data of pharmacokinetics and biodistribution which strongly suggest the micelle formation of PEO/PASP (ADR) in the body compartments, preventing the unwanted disposition and the initiation of toxic events. Also, micelle associated ADR may express toxicity differently than ADR.

One should use caution in applying the synthetic polymers as drug carriers because of the chronic toxicity due to non-specific accumulation in the body [1]. It is an unfortunate characteristic of polymeric drugs that the increasing half-life by increased molecular weight often causes increased toxicity due to non-specific accumulation. It is further of interest to note that polymeric micelles may overcome this and achieve both sufficient in vivo half-life and low accumulating toxicity by regulating the micelle dissociation as illustrated in Fig. 4. As mentioned previously, micelles may decay through the dissociation of the constituent block copolymers (unimers). It is likely that change in the physicochemical properties of the unimer through drug release, partial degradation or environmental variation, for example, may accelerate micelle dissociation. Then, unimers with relatively low molecular weight (ca. 10 000) can smoothly excrete from the kidney. Consequently, by regulating this dissociation process, it is feasible to balance the retention and the excretion of polymeric micelle drugs in the body. In contrast with a prompt dissociation of micelles of short-chain surfactants in non-equilibrated condition, the dissociation rate of a unimer from polymeric micelles is considered to be low enough to compete with the distribution rate of polymeric micelles in the body compartments. Another critical issue in drug-carrier conjugate is that drugs conjugated to carrier matrices may be treated as haptens inducing immune responses. Polymeric micelles may overcome this by utilizing PEO as an outer shell. As is well-known, chemical modification of antigenic proteins with PEO drastically diminishes the immunogenicity of the proteins [42–45] allowing their repetitive clinical usage.

Recently, i.v. injection of PEO/PASP (ADR) was found to be quite effective for the treatment of several solid tumors subcutaneously transplanted and massively developed in animals [32]. Worth noticing is that PEO/PASP (ADR) showed even superior tumoricidal activity than its parental drug, ADR. As shown in Fig. 5, quite impressive results were obtained for murine colon adenocarcinoma 26 (C26) in that the tumor completely disappeared after i.v. injection of PEO/PASP (ADR); while only a partial inhibition of tumor growth was achieved by ADR. The fact that PEO/PASP (ADR) expressed superior antitumor activity to its parent drug surely confirms the promising feature of vehicle design for the enhancement of the tumoricidal index of conventional anticancer drugs through a varia-

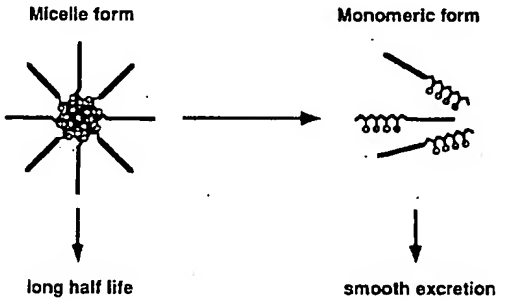

Fig. 4. Feasible balance of the retention and the excretion of polymeric micelle drugs in the body through dissociation control.

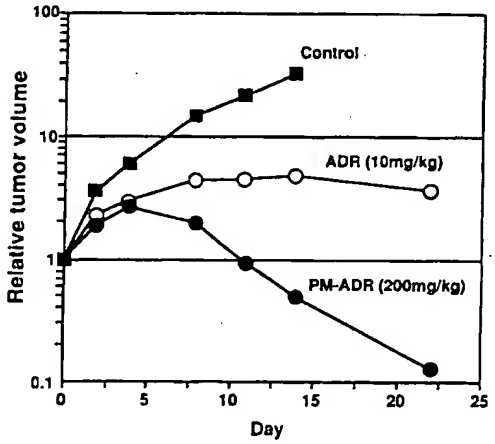

Fig. 5. In vivo anti-cancer activity against mouse adenocarcinoma (Colon 26). Tumor bearing mice were given i.v. injection (3 doses at 4 day intervals) of free ADR or polymeric micelle drug (PM-ADR) at optimum dosage.

tion in pharmacokinetics and biodistribution of parental drug. We have recently verified that the in vivo antitumor activities of PEO/PASP (ADR) were strongly dependent on their composition, while their in vitro cytotoxicities were almost similar for all the compositions [53]. This result strongly suggests that in vivo activity of PEO/PASP (ADR) might depend on its tumoritropic efficacy, which is crucially varied with the composition of PEO/PASP (ADR) because of the change in the properties of the micelles thus formed. Indeed, PEO/PASP (ADR), with an appropriate composition to achieve longer circulation in blood, was found to show highest anticancer activity toward solid tumors in mice [52].

Excellent tumoricidal activity of PEO/PASP (ADR) against extravascular tumors suggests that there seems to be a promising capability of extravasation in this system. It is an important facet of this article to note the possible utility of polymeric micelles for delivering a drug to extravascular targets, especially solid tumors. It might be reasonable to consider polymeric micelle transport directly through the tumor vasculature to the tumor tissue, because a massively developed tumor shows an extensive neovasculature development with enhanced vascular permeability [54]. Further, tumor tissue is known to have a poorly developed lymphatic system, resulting in a decreased recovery of macromolecular compounds via the lymphatic system [55]. Indeed, as beautifully demonstrated by Matsumura and Maeda [56], macromolecular anti-cancer drugs (SMANCS) tend to show a predominant accumulation in solid tumors. However, the efficacy of extravasation of particles in the range of around 50 nm, even though vascular permeability is considerably enhanced in tumor tissue [57,58] may be an important issue. In this context, it is important to recognize that polymeric micelles have soft-shell/hard-core structures in which the densely packed core is surrounded by a highly hydrated and flexible shell. When the solvent compatible block length (i.e., length of the shell-forming segment) is considerably larger than that of the incompatible block (i.e., length of the core-forming segment), we can assume the star model proposed by Halperin [59] utilizing a scaling theory for the micelle structure in which the PEO chains in the corona are in a semi-diluted condition with a decreased concentration (or increased hydration) profile along the radius. Consequently, the shell has a considerable elasticity which may induce a shape change in the micelle. This dynamic feature of the micelle may facilitate its penetration through the vessel wall and interstitial space in the body.

An alternative to the direct extravasation of the micelle as discussed above, is that tissue penetration of the unimer might also be possible by regulating the dissociation rate of the unimer from the parental multimolecular micelles. It is to be noted that a unimer with considerably long hydrophilic blocks may form monomolecular micelles to decrease interfacial free energy [10,11]. These monomolecular micelles are smaller in size than parental multimolecular micelles, and thus are expected to have a higher diffusivity through tissue.

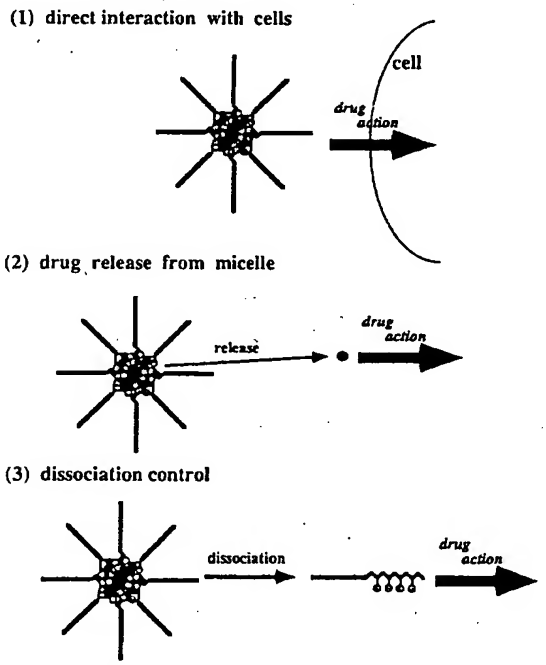

Fig. 6. Action mechanisms of micelle-forming polymeric drugs.

Finally, we have to address the possibility of utilizing polymeric micelles as depots for drug release. This approach is promising, not only because of the intravascular drug release but also because of the release of drugs in the extravascular compartment subsequent to the extravasation of the micellar vehicle. In summary, three major mechanisms involved in the polymeric micelle drug action are shown in Fig. 6. Of course, second and third action mechanisms are able to be realized in both extra- and intra-cellular situations.

Conclusion and future perspectives

As described in the Introduction, natural vehicles with supramolecular structure (viruses and lipoproteins) have advantageous features summarized as: (1) formation of environmentally separated microcontainers through supramolecular assembly: (2) biocompatibility or biological stealthiness; (3) installation of an anchoring moiety on the surface (spatial or site recognition); (4) time- and site-dependent stability (chronological and environmentally-responsive action). These features can be mimicked at least to some extent by synthetic block copolymer micelles with a further advantage of higher feasibility in structural tailoring. As the horizon of polymer synthesis is extensive, and is still expanding, we may readily imagine the development of polymeric micelle drugs with many distinctive patterns of action. This article mainly featured polymeric micelles with conjugated anticancer drugs. An alternative to this conjugated-type, stable entrapment of drugs in the inner core through physical binding is possible. In this context, the balance between the stability and the solubilizing power of the micelle is quite important. A rationale is required for the structural design of the inner-core segment in terms of length, chain mobility, the nature of cohesive force and biocompatibility. Recently, our research group has found the formation of a stable micelle with a cmc of 10 mg/l for the block copolymers of poly(ethylene oxide) and poly(β-benzyl-L-aspartate) [60], opening a way of preparing micelles with poly(amino acid) cores which may have a potential utility in drug delivery systems. Concerning the installation of pilot molecules (targeting moieties), challenging work has been done by Kabanov et al. [19] using a poloxamer conjugated with an antibody molecule at the chain end. They reported an increase in the in vivo neuroleptic action of haloperidol intraperitoneally injected into mice in a micellar solution of poloxamer with conjugated antibodies specific to brain [19], although physiological micelle formation was not demonstrated. Previously, we have reported a conjugation of PEO/PASP (ADR) with immunoglobulin G (IgG) utilizing the terminal -NH_2 group of PASP segment [29,30]. By the use of *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) as a coupling reagent, disulfide linkage was introduced between PEO/PASP (ADR) and IgG. As schematically shown in Fig. 7, this conjugate formed a unimolecular micelle-like structure, in which the PASP (ADR) segment associates around the disulfide bond. Consequently, the disulfide bond became stable enough to resist degradation by dithiothreitol, unless sonication was applied [61]. This extraordinary stability of the disulfide linkage between IgG and the block copolymer is expected to contribute greatly to the stabilization of the conjugate in vivo, and may increase the targeting efficiency of the conjugate. Further, the PEO segment in the conjugate contributes to an increase in the solubility of the conjugate as well as preventing possible biologi-

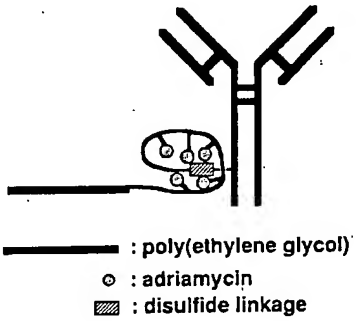

Fig. 7. Immuno-conjugates with stabilized disulfide linkage.

cal side-effects, including immune responses, complement-activation and short circulating life. These results surely suggest many fascinating applications of polymeric micelles with targeting moieties.

Finally, we should focus on the behavior of polymeric micelles at the cellular interface. This is a very fundamental matter required for revealing the mechanisms involved in the action of micelle-forming polymeric drugs. To our knowledge, the only work to have appeared in the literature concerning this subject was by Llyod et al. [21]. They observed a considerable uptake of the amphiphilic block copolymer by rat peritoneal macrophages in vitro through pinocytosis. As described in previously, PEO shows anomalous features in its aqueous entity. In diluted conditions, PEO chains are in a highly hydrated state with a large exclusion volume, leading to very effective steric repulsion [45,62]. However, in a concentrated state, they are considered to show rather attractive interactions with each other, or with components, directly through hydrophobic interaction or indirectly through the bridge of bound water molecules. A recent detailed study using TEM revealed considerable protein adsorption on PEO-grafted surfaces when in contact with blood for a prolonged period in vivo [63]. Further, it is well-known that PEO can serve as proton-accepting polymer to form a complex through hydrogen bonding with a proton-donating polymer such as poly(acrylic acid) [64]. These results lead to the consideration that intermacromolecular interaction may occur between the plasma membrane and the PEO chain of the micelle shell due to a highly local concentration of macromolecules at the interface. This mode of interaction crucially influences the fate of the micelle at the target site where the long-term and chronological interactions of micelles with target cells take place. In this context, more intensive efforts should be devoted to this area of research to establish polymeric micelles as one of the major vehicles in the field of site-specific drug delivery.

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